Bulgarian Journal of Veterinary Medicine, 2022 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2022-0089

Original article

INVESTIGATING DIFFERENTIAL EXPRESSION GENES PROFILE IN HCT-8 CELL LINE INFECTED WITH *CRYPTOSPORIDIUM PARVUM* IN HOST-PARASITE INTERACTIONS

S. DADKHAH TEHRANI[1], S. R. SHOJAEI[1], S. R. HOSSEINI[2] & P. SHAYAN[3]

[1]Department of Parasitology, Karaj Branch, Islamic Azad University, Karaj, Iran; [2]Department of Parasitology, Shahr-e-Kord Branch, Islamic Azad University, Shahr-e-Kord, Iran; [3]Department of Parasitology, University of Tehran, Tehran, Iran

Summary

Dadkhah Tehrani, S., S. R. Shojaei, S. R. Hosseini & P. Shayan, 2022. Investigating differential expression genes profile in HCT-8 cell line infected with *Cryptosporidium parvum* in host-parasite interactions. *Bulg. J. Vet. Med.* (online first).

Cryptosporidium parvum is a microscopic parasite and furthermore, an identified agent that spends its life cycle in the mammalian gastrointestinal tract causing chronic and life-threatening diarrhoea in immunocompromised individuals. In this study, the GSE2077 series were selected from the NCBI site, which examined the contamination of the HCT-8 cell line with *C. parvum* in three treatment groups. Each of 24, 48, and 72 hours post-infection (PI) groups was compared with the mock, and the differentially expressed genes (DEGs) were identified during the analysis. For each comparison, the |logFC|= 2 and P values <0.05 were considered. The obtained values included: 24 hours=71 DEGs, 48 hours=82 DEGs, and 72 hours=55 DEGs. For the DEGs of each group, gene ontology diagrams were drawn separately using the Funrich3.1.3 software, including cellular components, biological processes, and molecular functions. The heat map diagrams were drawn with the R software and the heat map package. Also, the networks were plotted for each comparison in the Cytoscape software, and hub genes were obtained. Finally, the commonalities between the three treatment groups were identified using the FunRich software. Five common genes were revealed in all groups: *RAD23B, DKK1, CXCL8, PHLDA1*, and *UGT1A3*.

Key words: *Cryptosporidium parvum,* cryptosporidiosis, intestinal epithelium, HCT-8 cell line, parasite infection

INTRODUCTION

Cryptosporidium parvum is one of mammalian most important enteric parasites, especially in domestic animals and humans. Some *Cryptosporidium* species have a specific host, but *C. parvum* is infectious for all mammals and sometimes can infect several host organs. *Cryptosporidium* is found in all parts of the gas-

trointestinal tract, especially in the villi of the intestinal wall, causing clinical symptoms. Clinical infection in mammalian infants and childrens is more common and severe than in adults. Cryptosporidiosis leads to acute or chronic gastroenteritis. It is observed in both clinical and subclinical forms in humans and animals and is associated with many complications. The disease symptoms are almost identical in humans and animals, but their incubation period is different (Gharekhani *et al.,* 2014; Yasur-Landau *et al.,* 2021).

With the discovery of AIDS in the early 1980s, this parasite became critical because it was one of the most important causes of severe, long-term, and life-threatening diarrhoea in these patients. The parasite can be transmitted to humans through contaminated food and water or personal or animal contact. Pathogens affect host cellular mechanisms to direct host cell capabilities in the path of infection (Chawla *et al.,* 2011; Awulachew *et al.,* 2020). From the view of cellular genetics, it should be noted that human intestinal cells undergo significant changes in host-parasite interaction, especially changes in the expression of relevant genes due to *C. parvum* infection. It also examines the changes parasites undergo during the transition from one stage of life or from one host to a new one, defines biochemical pathways necessary for organisms to survive in the host, and emerges new treatment strategies. Understanding the host and pathogen interaction is essential for identifying the mechanisms of infection. Following the advancement of technology over the past decade and the availability of different levels of Omics data collocated from pathogens, general information is provided for researchers on pathogen-host reactions, biological system-based methods to infer and analyse regulative, immune, metabolic, and protein-protein networks, and host-pathogen interaction to identify and elucidate the mechanism of infection.

Castellanos-Gonzalez *et al.* (2008) cultured human ileal mucosa for infection with *C. parvum* and *C. hominis* to determine which genes were regulated during infection and analysed gene expression by microarray assay. They determined that osteoprotegerin (OPG) mRNA protein was produced when the HCT-8 cells were infected with the parasite. Also, the authors demonstrated that epithelial cell apoptosis was induced, and parasite number was reduced when infected cells were treated by OPG ligand tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). So these results indicated a new pathway for eradicating *C. parvum* infection. In another study, Borowski *et al.* (2010) inoculated *C. parvum* into the HCT-8 cell line and for the first time evaluated the host-parasite interaction with scanning electron microscopy. Liu *et al.* (2018) analysed the expression profile of long non-coding RNA (lncRNA) and mRNAs in the HCT-8 cells infected with *C. parvum* by microarray assay and revealed that differently expressed lncRNA played an essential role in infection regulation.

The importance of cryptosporidiosis as a zoonotic disease in public health and a life-threatening factor in AIDS patients prompted us to study the genes expressed in host-parasite interactions differently than other studies done in the past and using bioinformatics tools. The present study will introduce common genes having the most changes in expression at 24, 48, and 72 hours PI as potential targets for the drug and vaccine industry. However, more research is needed in the future for this purpose.

MATERIALS AND METHODS

Microarray analysis

Initially, *C. parvum* parasite-related Omics studies were reviewed. The keywords such as HCT-8 cell line, *C. parvum* and human infection with it were used in the GEO database. Appropriate data were selected, and unrelated data were deleted. Finally, the gene expression dataset (GSE2077 series), which examined the contamination of the HCT-8 cell line with *C. parvum* in 18 samples, was downloaded from the GEO database. This study's samples were divided into four groups: control group, 24 hours PI group, 48 hours PI group, and 72 hours PI group.

Classification of groups

The active groups were classified as pairs to analyse the DEGs. For this purpose, three binary groups were created: 1) Control group versus 24 hours PI group; 2) Control group versus 48 hours PI group; 3) Control group versus 72 hours PI group.

After pairing the involved groups and placing the studied groups in them, they should become an expression set. R software, GEOquery, Biobase, and Limma packages were used. The Boxplot package in R software was used to control the expression sets. Data were normalised with normalise between arrays. After vertical integration of the DEGs, the studied groups were divided into three mentioned above to find commonalities. Each of them was placed in a Venn diagram to obtain the similarities and differences of the genes resulting from vertical integration (Fig. 1).

After vertical integration, the genes obtained in the three groups were considered DEGs and used to draw the mother

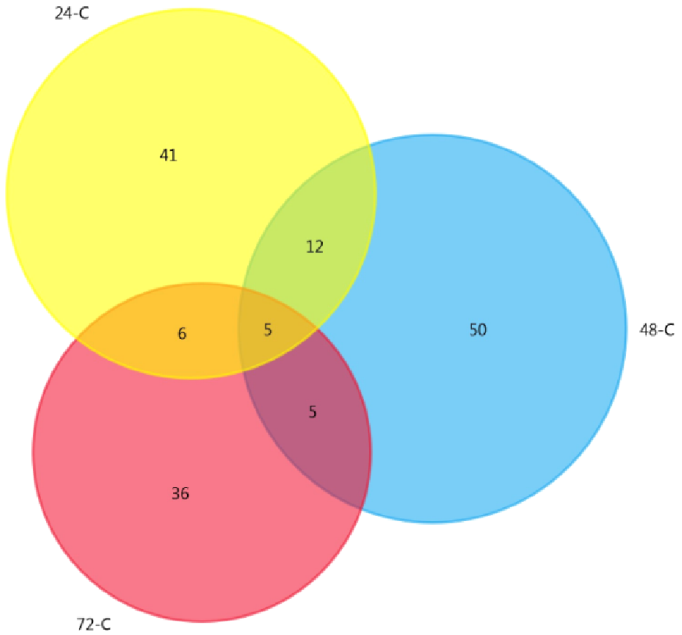

Fig. 1. Venn diagram for each 24,48, and 72 hours PI group compared to the control group; C = control.

network. It was constructed using the Search Tool for the Retrieval Genes and Protein (STRING) database. The drawn mother network was analysed using Cytoscape software, and network analyzer application and centrality criteria were determined for all three nodes.

The Gephi software was used to find modules in the network and the main and essential processes and functions. Different algorithms containing different parameters were used in each of these analyses, and optimisation of these parameters is one of the most critical points of this phase. After converting the obtained networks into three groups, all three networks were divided into several logical modules. The best module was selected and introduced based on gene-protein enrichment analysis.

Gene enrichment analysis methods were used to find the function of unknown genes/proteins or the specific role of known genes/proteins with multiple functions. All these methods are data annotation in Gene Ontology (G.O.) and Kyoto Encyclopedia of Genes and Genomes (KEGG). For this purpose, the biological pathways and involved networks in the cellular signalling pathways of the obtained genes were introduced in the module. Each of the three groups for the gene-protein enrichment process was placed on the Enrichr site.

RESULTS

Essential genes with the highest centrality indices were selected and introduced based on the results of the Venn diagram in three main groups, vertical integration of obtained DEGs, analysis of network centrality, obtained hubs, and degree index (Table 1).

Table 1. The list of top 10 genes based on degree index for network drawing in three groups.

Rank	Name	Score
First group (24 hours PI without control group)		
1	*GAPDH*	13.0
2	*CXCL8*	8.0
3	*IGF2*	6.0
4	*ACTB*	5.0
5	*RAD23B*	4.0
5	*TEK*	4.0
7	*ABCC2*	3.0
7	*SLC4A4*	3.0
7	*SLC15A1*	3.0
7	*GNB2L1*	3.0
Second group (48 hours PI without control group)		
1	*IL6*	21.0
2	*GAPDH*	19.0
3	*CXCL8*	14.0
4	*FOS*	13.0
5	*CTGF*	11.0
6	*HGF*	10.0
7	*PTPN11*	9.0
8	*MYB*	7.0
9	*AGTR1*	6.0
9	*FGF4*	6.0
Third group (72 hours PI without control group)		
1	*IL6*	6.0
2	*PTPN11*	5.0
3	*DKK1*	3.0
3	*LEF1*	3.0
5	*CXCL8*	2.0
5	*IL1RN*	2.0
5	*TNKS*	2.0
8	*MSTN*	1.0
8	*LILRB1*	1.0
8	*ACADL*	1.0

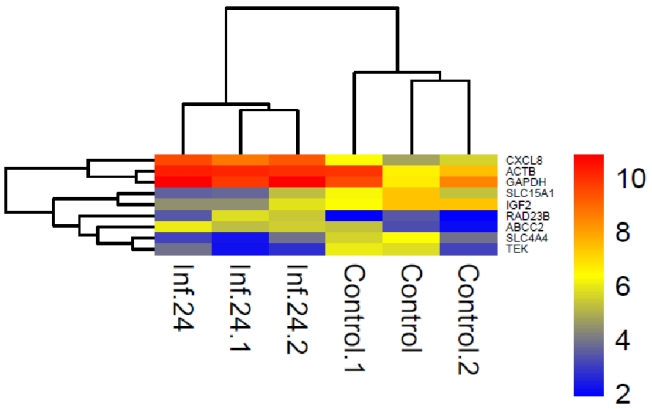

Fig. 2. Heat map diagram for the first group (24 hours PI without control group).

24 hours PI in the *C. parvum* infected HCT-8 cell line, and in the discussion of biological processes, 27.6% of mostly expressed genes were associated with cell-cell interaction. Of these genes, 1.7% were related to activities that inhibit cell death, and 1.7% were associated with regulating the conduction of cellular messages. Those involved in protein metabolism, cell transfer, and cell-binding processes were 13.8%, 13.8%, and 3.4% respectively. Exosomes are the essential extracellular vesicles that play a crucial role in cell-cell communication.

Cellular components of 24 hours PI DEGs showed that 4.5% of genes were involved in peroxisome and its activity. Among the most expressed genes, 16.1% and 1.8% were involved in plasma membrane correction and the cytoplasmic cyclin-dependent protein kinase holoenzyme complex. Glycine-influenced functions related to the chloride channel complex were applied by 1.8% of the mentioned genes. Of the genes discussed above, 28.6% affected extracellular activities and 5.4% of them mainly affected the basal and sides parts of the plasma membrane. In the heat map diagram discussion, *GAPDH*, *ACTB*, and *CXCL8* genes were more expressed in the treatment groups than in the control groups. *TEK* and *SLC4A4* expressed in the treatment were reduced compared to the control groups (Fig. 2).

KEGG pathway analysis in this time shift demonstrated that the DEGs were involved in activating the pathways related to bile secretion, proteoglycans in cancer, Rap1, phospholipase D, Hippo, and MAPK messaging pathways, ABC vectors, shigellosis disease, improper transcriptional regulation in cancer, and cell connections.

Molecular functions of the DEGs in HCT-8 cell line infected with *C. parvum* in 24 hours PI showed that 1.7% of carboxylase activity was affected. Of these genes 3.4% appeared to act as protease inhibitors, 1.7% involved adenylate cyclase activity, 12.1% of them affected transcription factor activity, 5.2% influenced membrane receptor protein activity and membrane processes related to tyrosine kinase activity, and 10.3% were involved in protein transfer activities.

In the top 10 genes network study for the first group (24 hours PI without the control group), *GAPDH*, *ACTB*, *CXCL8*, and *IGF2* genes had the highest and most

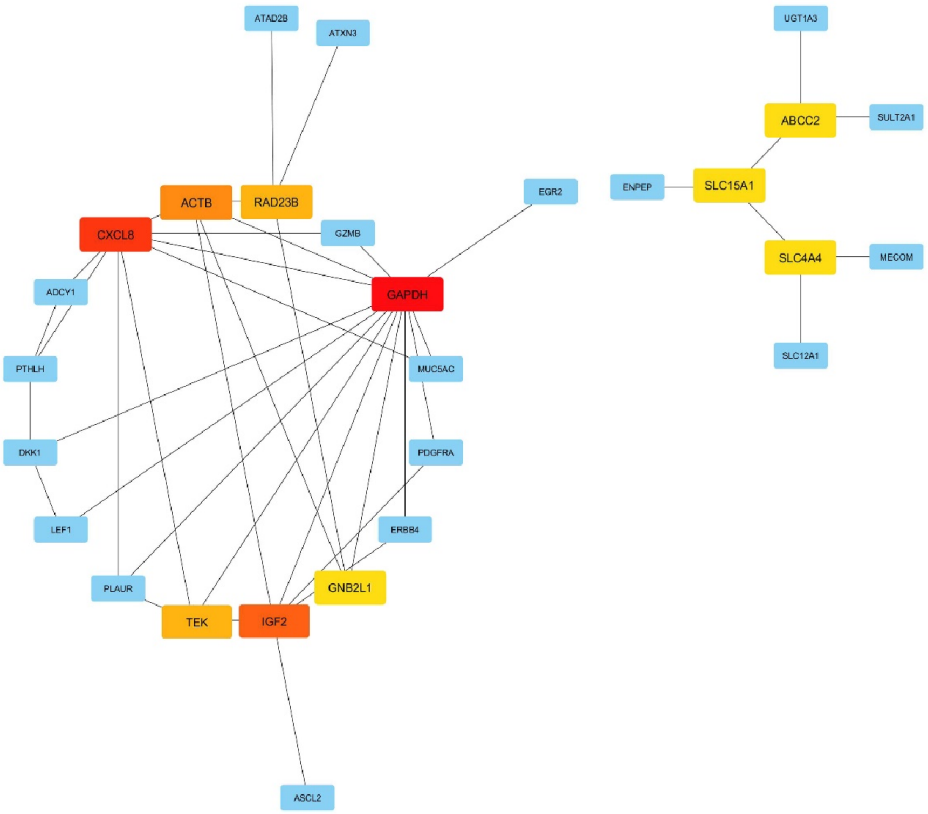

Fig. 3. Top 10 genes network for the first group (24 hours PI without control group).

significant expression impact on biological activity among other genes in this shift time (Fig. 3).

Of the genes associated with cellular homeostasis, 1.5% were involved in cell deformation during cell differentiation, 1.5% in regulation of blood pressure, 1.5% in hormone metabolism, and 39.7% in message transmission. Moreover, 39.7% of these genes were involved in cellular communication, significantly affecting cellular activities (P<0.05).

Of cellular components at 48 hours PI, 1.6% of genes were related to the density of filaments forming of platelet network, 1.6%, 3.2%, 3.2%, and 11.1% were involved in the formation of microtubules, discussion of the basement membrane, vesicles bound to the plasma membrane, and in extracellular space, respectively. Overall, 31.7% of those were involved in extracellular processes, whose change was significant (P<0.05). On the heat map diagram, *IL6, MYB, PTPN11, FGF4, CXCL8, CTGF*, and *FOS* genes were more expressed in the treatment groups than in the control. The expression of the *AGTR1* gene in the treatment groups was reduced compared to the control group (Fig. 4).

Molecular functions of DEGs in the HCT-8 cell line infected with *Cryptosporidium* in this time shift comprised 1.5% of genes that were involved in the

protein enzyme tyrosine phosphatase activity, 2.9% considered receptors for cell binding, 8.8% involved in transfer activities, 4.4%: in cytokine activities, 13.2%: in transcription factor activity, and 5.9% - in growth factor activity.

In the top 10 genes network study for the second group (48 hours PI without control group), *HGF, FOS CTGF, CXCL8, GAPDG*, and *IL6* genes had the highest expression compared to other genes (Fig. 5).

Of these genes with anti-apoptotic activity 17%, 2.1%, 17%, 2.1%, and 10.6% were involved in metabolism, controlling cell proliferation, pathways related to cell energy supply, calcium ion messaging as a mediator, and immunity responses, respectively.

Cellular components of DEGs 72 hours PI showed that 2.2% of genes were involved in the debate of proteins involved in DNA complex, 6.7% and 11.1% of them were engaged in extracellular spaces, 4.4% of those were related to other proteins, 2.2% of these genes were associated with IL6 receptors, and 2.2% of them were involved in collagen formation.

LEF1, DKK1, IL1RN, PTPN11, CXCL8, IL6, L1LRB1, TNKS, and *MSTN* genes were more expressed in the heat map diagram discussion in the treatment groups than in the control groups. The expression of the *ACADL* gene in the treatment groups was reduced compared to the control group (Fig. 6).

Molecular functions of DEGs in the HCT-8 cell line infected with *C. parvum* in 72 hours PI were as followed: 4.3% and 2.1% were considered receptors for cell binding and a specific protein for binding, 4.3% were involved in growth factors activities, 10.6%, 14.9%, and 6.4% of genes were involved in catalytic, transcription factors, and cytokine activity.

DISCUSSION

Cryptosporidium is the primary cause of diarrhoea in children and untreated AIDS patients in underdeveloped countries (Dumaine *et al.,* 2020). In recent years, advances in genomics, epigenetics, and

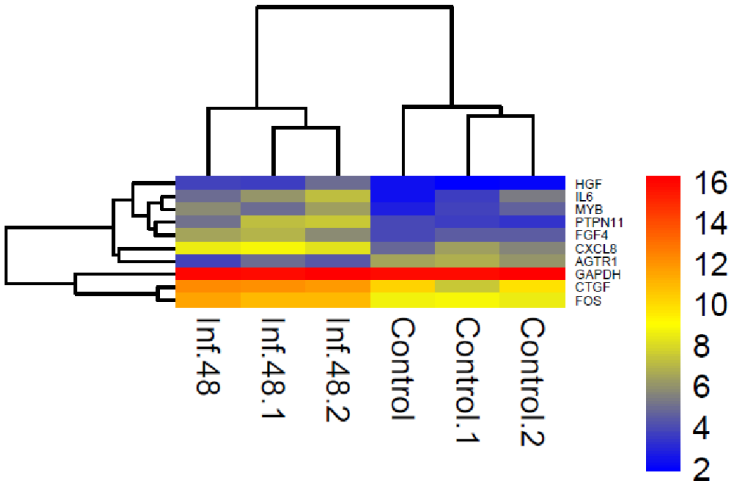

Fig. 4. Heat map diagram for the second group (48 hours PI without control group)

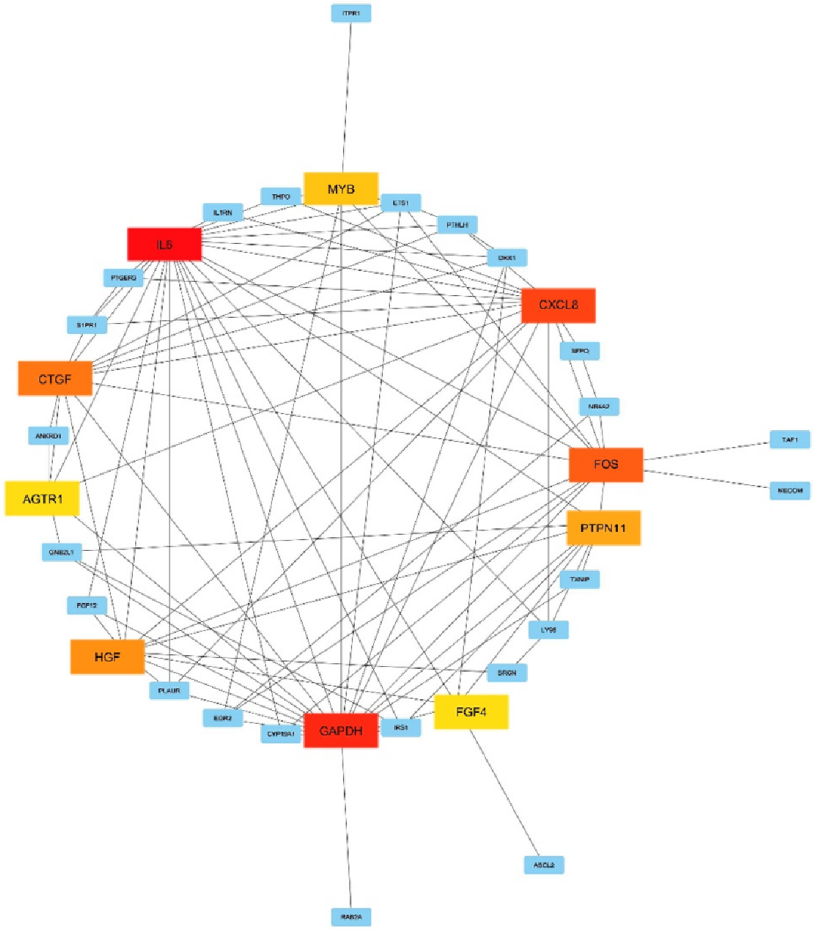

Fig. 5. Top 10 genes network for the second group (48 hours PI without control group).

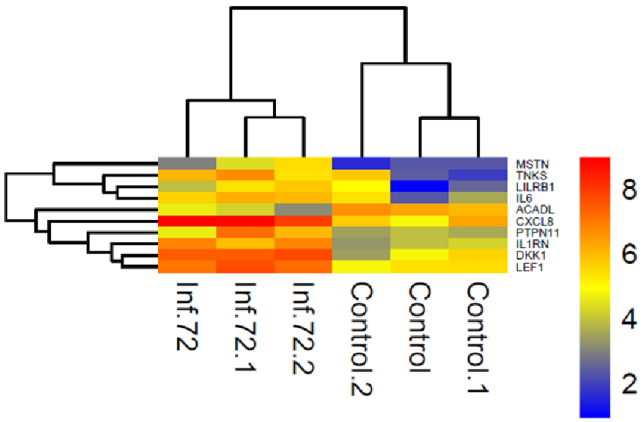

Fig. 6. Heat map diagram for the third group (72 hours PI without control group).

transcriptomics have led to comprehensive analyses of the molecular mechanisms involved in developing various diseases. Along with these developments, many databases have been created containing multiple biological data. Like GEO, most databases provide their data to researchers for free. Much of these data can be processed by bioinformatics tools and other network analyzer software to investigate the molecular and biological mechanisms involved in the progression of various diseases. The science and technology of microarray are widely used to analyse and measure omic changes, including proteomics, genomics, and gene expression. Microarray data can provide researchers with accurate results for laboratory analysis. Therefore, genomics, transcriptomics, and proteomics data can be combined to obtain meaningful and appropriate consequences. When the omic layers are merged, a correct understanding of biological systems can be achieved. Recognition and understanding of the complex network involved in the pathogenic process of the protozoan and identification of the most critical factors involved in this process led to the design of new diagnostic methods, use of appropriate drugs to target essential factors that the protozoan uses to change cellular processes and its proliferation in a host, and also the design of a proper vaccine.

Liu *et al.* (2009) examined changes in apoptosis at different times PI (6, 12, 24, 48, and 72 hours PI) in the *Cryptosporidium*-infected HCT-8 cell line. They found that 333 genes were altered at least two of the five mentioned times during transcription. Fifty-one regulated genes were related to apoptosis and divided into five categories based on their expression patterns. In early and late infection, anti-apoptotic genes were up-regulated and down-regulated, respectively. Also, in early and late disease, apoptotic and pro-apoptotic genes were down-regulated and induced, respectively. They demonstrated that the first apoptosis occurred at 12 hours PI and increased within 24 hours. The results showed that the experimental silencing of Bcl-2 caused apoptosis in 505 infected cells within 24 hours after illness, which reduced the accumulation of meront-containing cells.

Moreover, they inhibited apoptosis using a pan-caspase inhibitor, which could effectively treat the *Cryptosporidium* infection. To better understand the host response to *C. parvum* infection, Deng *et al.* (2004) examined the gene expression profile of infected human ileocecal adenocarcinoma cells using Affymetrix oligonucleotide microarray containing probe sets for 12600 human genes. The results showed that the 223 identified genes, including 125 up-regulated and 98 down-regulated, were regulated with this parasite infection 24 hours PI. Thirteen of these genes were analysed by qRT-PCR. Host heat-shock genes and pro-inflammatory chemokines IL-8, RANTES, and SCYB5 were revealed. Finally, this study determined that the parasite leads to dramatic changes in host biochemical pathways and offered new insights into specific biological functions of infectious diseases caused by an intracellular protozoan parasite.

Expression of 3,302 genes was studied in *C. parvum*-infected cell over 72 hours (Mauzy *et al.,* 2012). The parasite had detectable transcription of all genes *in vitro* within at least one time point tested, and adjacent genes were not co-regulated. Five genes were not detected within the first 24 hours of infection, containing two AP2 domains as a transcription factors. The fewest genes detected were at 2 hours

PI. In contrast, 30% of the genes had their highest expression at 48 and 72 hours PI. The protein involved in translation became over-expressed in 6 hours, while the structural proteins were over-expressed in 12 hours. These changes confirmed the altered gene expression in the parasite-infected HCT-8 cell line.

Studies have shown that the expression of genes involved in HCT-8 cell line infection is affected by *C. parvum*. In the process of disease development and at different time shifts, gene expression is affected so that gene expression shows different values of 48 hours PI and 72 hours PI. Increased or decreased expression of genes due to infection causes changes in biological processes and activation of cellular biological pathways. Each of them alone or cooperates with other pathways or factors activates diseases or other functions that are not necessarily pathogenic.

The cellular communication pathways, investigated in our study, were characterised by Hu *et al.* (2013) in *C. parvum*-infected epithelial cells. The authors showed that this parasite could increase exosome production from these cells and suggested that exosomes derived from parasite-infected epithelial cells induced TLR-4 pathway inflammation against the parasite. In addition, it was suggested that exosomes derived from infected epithelial cells may increase the expression of pro-inflammatory genes in splenic white blood cells (Wang *et al.,* 2019). Studies showed also that these exosomes contained antimicrobial peptides such as cathelicidin-37 and β-defensin-2. Due to the location of the parasite around the host cells, the results of our study become more reliable due to the enrichment of most genes in extracellular activities. Our study was not the only bioinformatics analysis in which the resulting genes are enriched in this way. However, the data analysis study of O'Connor *et al.* (2009) showed the same result.

Our studies showed that bile salts are one of the main elements of *Cryptosporidium* secretion from its oocysts and metabolism, temperature, pH, and reducing factors. King *et al.* (2012) showed that bile salts, in addition to accelerating the parasite from its oocytes, increased also the invasion of parasitic sporozoites. To determine the proteins the parasite needs for invasion, it was shown that the ABC vectors were essential for feeding the parasite in host cells (Singh *et al.,* 2015). Munoz-Caro *et al.* (2015) demonstrated that the MAPK, induced by the parasite and increased its expression, was one of the main factors in the formation of NETosis.

Increasing the expression of transcription factors may indicate that the parasite's entry into epithelial cells can trigger the expression of various genes, including pro-inflammatory ones, production of cytokines, and antimicrobial peptides genes.

At 48 hour PI in the HCT-8 cell line infected with *C. parvum* and in the discussion of the biological process, 1.5% of these mostly expressed genes were associated with cellular homeostasis.

Comparison of DEGs enrichment at 48 hours PI to 24 hours PI meant that infected cells at these time intervals tried quickly to communicate with each other and improve growing conditions, while the involvement of genes in cellular communication had increased from 27.6% to 39.7% in the 48 hours PI.

As mentioned, due to the location of this parasite in the extracellular space, it can be concluded that the DEGs in in-

fected cells at 24 hours PI were also enriched in the extracellular pathway.

KEGG pathway analysis in this time shift demonstrated that the DEGs were involved in activating the pathways in cancer, pertussis disease, Toll-like, type C lectin, and adipocytokine receptors signalling pathway, parathyroid hormone production and involvement in its secretion and action, malaria disease, kidney cancer, thyroid hormones production, and aging cells. The expression of the TLR-4 gene, an essential protein for inhibiting the innate immune response and producing pro-inflammatory cytokines, was significantly increased in intestinal epithelial cells (Zaalouk *et al.,* 2020). In addition, this increase in expression was mediated by miRNA (Chen *et al.,* 1993). The study demonstrated that reducing the expression of miR-let-7i and thus reducing the suppression of the *TLR-4* gene increased the expression of this gene in infected and epithelial cells. Increasing TLRs stimulation can increase the secretion of cytokines such as IL-8, TNFα, and GROα in basolateral cells (Crawford *et al.,* 2021). The secretion of the IL-6 cytokine, a cytokine enhancing the differentiation of T-cells to TH17, suggests that epithelial cells play a significant role in simulating acquired immunity against this parasite.

The role of *HGF, FOS CTGF, CXCL8, GAPDG*, and *IL6* genes in the time shift, observed in our study, was more prominent. Increased expression of genes related to transfer protein can also be interpreted as metabolic utilisation of host and parasite-infected cells by the parasite.

At 72 hours PI period in the HCT-8 cell line infected with *C. parvum* and in the discussion of biological processes, 2.1% of mostly expressed genes were associated with anti-apoptotic activity. This parasite significantly affected infected cells' metabolic shift and energy and altered the host cells' nucleotide metabolism using a porine vector (Pawlowic *et al.,* 2019). It also demonstrated that the host cell's metabolism changed 72 hours PI.

The decrease in genes involved in extracellular activities and the increase in genes related to intracellular activities can be due to the transfer of the parasite from the extracellular space to the intracellular space.

KEGG pathway analysis in this time shift demonstrated that the DEGs were involved in activating the effective pathways in amoebiasis, AGE-RAGE signalling pathway in diabetes, phospholipase D signalling pathway, non-alcoholic fatty liver disease, interaction between cytokines through target receptors, malaria and legionellosis, steroid hormone production, NOD-like signaling pathway receptor, and improper regulation of transcription in cancer.

In the top 10 genes network study for 72 hours PI group, *DKK1, PTPN11, IL6*, and *LEF1* genes had the highest expression and the most significant impact on biological activity among other genes in this time shift (Fig. 7).

The commonalities between all the significant genes that had the greatest impact on the cell studies (using the Venn diagram) revealed five genes common to all of these states: *CXCL8, DKK1, RAD23B, UGT1A3*, and *PHLDA1*. *DDK1*, *PHLDA1*, and *CXCL8* had increased expression in *Cryptosporidium*-infected cells among the common genes (Fig. 1). Ming *et al.* (2018) showed that the *DKK1* gene could be one of the essential genes for the parasite life cycle inside host intestinal cells and also be one of the candidates for *Cryptosporidium* treatment. The *PHLDA1* gene was introduced as one of

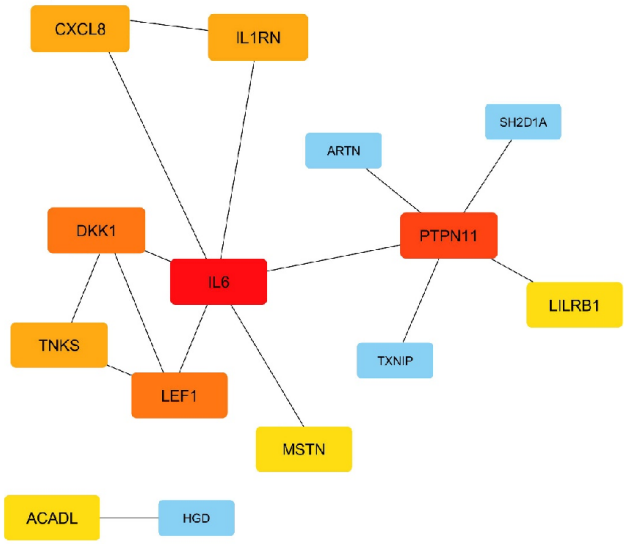

Fig. 7. Top 10 genes network for the third group (72 hours PI without control group).

the genes involved in anti-apoptotic (Liu *et al*., 2009). Laurent & Lacroix-Lamandé (2017) introduced the expression of the *CXCL8* gene as a factor in increasing the accumulation of neutrophils in the intestinal epithelium of patients with cryptosporidiosis.

CONCLUSION

This study showed that most of the genes involved in infected cells depended on immunological pathways such as cytokine IL-6 and chemokine CXCL8 expression. In addition, the routes of bile salts production, metabolic pathways; extracellular, intracellular, and anti-apoptotic activities were introduced as pathways involved in *Cryptosporidium* infection at different times (24, 48, and 72 hours post infection). Finally, the *RAD23B* and *UGT1A3* genes, which were the final genes and were not studied by other researchers, will be presented as target genes to treat cryptosporidiosis in future studies.

ACKNOWLEDGEMENTS

We would like to thank the Department of Parasitology of the Faculty of Veterinary, Islamic Azad University Karaj, for their help conducting this study.

REFERENCES

Awulachew, E., K. Diriba, A. Gemede & F. Wudneh, 2020. Prevalence of cryptosporidium species among HIV/AIDS patients in Sub Saharan Africa; systematic review and meta-analysis. *Journal of HIV for Clinical and Scientific Research*, **7**, 006–012.

Borowski, H., R. C. A. Thompson, T. Armstrong & P. L. Clode, 2010. Morphological characterization of *Cryptosporidium parvum* life-cycle stages in an *in vitro* model system. *Parasitology*, **137**, 13–26.

Castellanos-Gonzalez, A., L. S. Yancey, H. C. Wang, B. Pantenburg, K. R. Liscum, D. E. Lewis & A. C. White Jr, 2008. *Cryptosporidium* infection of human intestinal epithelial cells increases expression of osteoprotegerin: A novel mechanism for evasion of host defenses. *The Journal of Infectious Diseases*, **197**, 916–923.

Chawla, R & R. Ichhpujani, 2011. Enteric spore-forming opportunistic parasites in HIV/AIDS. *Tropical Parasitology*, **1**, 15.

Chen, W., J. A . Harp, A. G. Harmsen & E. A. Havell, 1993. Gamma interferon functions in resistance to *Cryptosporidium parvum* infection in severe combined immunodeficient mice. *Infection and Immunity*, **61**, 3548–3551.

Crawford, C. K & A. Kol, 2021. The mucosal innate immune response to *Cryptosporidium parvum*: A global One Health Issue. *Frontiers in Cellular and Infection Microbiology*, **11**, 451.

Deng, M., M. S. Rutherford & M. S. Abrahamsen, 2004. Host intestinal epithelial response to *Cryptosporidium parvum*. *Advanced Drug Delivery Reviews*, **56**, 869–884.

Dumaine, J.E., J. Tandel, B. Striepen, 2020. *Cryptosporidium parvum*. *Trends in Parasitology*, **36**, 485–486.

Ebrahimzade, E., P. Shayan, S. Nabian & S. Rahbari, 2009. Identification of *Cryptosporidium parvum* by PCR and determination of its protein pattern and immunogenic antigens. *Journal of Veterinary Research,* **64**, 15–22.

Gharekhani, J., H. Heidari & M. Youssefi, 2014. Prevalence of *Cryptosporidium* infection in sheep in Iran. *Journal of Türkiye Parazitolojii Dergisi*, **38**, 22.

Hu, G., A. Y. Gong, A. L. Roth, B. Q. Huang, N. D. Hanson, X.M. Chen, H. D. Ward, G. Zhu & X. M. Chen, 2013. Release of luminal exosomes contributes to TLR4-mediated epithelial antimicrobial defense. *PLoS Pathogens*, **9**, e1003261.

King, B. J., A. R. Keegan, R. Phillips, S. Fanok & P. T. Monis, 2012. Dissection of the hierarchy and synergism of the bile derived signal on *Cryptosporidium parvum* excystation and infectivity. *Parasitology*, **139**, 1533–1546.

Laurent, F & S. Lacroix-Lamandé, 2017. Innate immune responses play a crucial role in controlling infection of the intestinal epithelium by *Cryptosporidium*. *International Journal for Parasitology*, **47**, 711–721.

Liu, J., M .Deng, C. A. Lancto, M. S. Abrahamsen, M. S. Rutherford & S. Enomoto, 2009. Biphasic modulation of apoptotic pathways in *Cryptosporidium parvum*-infected human intestinal epithelial cells. *Infection and Immunity*, **77**, 837–849.

Liu, T. L., X. C. Fan, Y. H. Li, Y. J. Yuan, Y. L. Yin, L. Zhang, X. T. Wang & G. H. Zhao, 2018. Expression profiles of mRNA and lncRNA in HCT-8 cells infected with *Cryptosporidium parvum* IId subtype. *Frontiers in Microbiology*, **9**, 1409.

Mauzy, M. J., S. Enomoto, C. A. Lancto, M. S. Abrahamsen & M. S. Rutherford, 2012. The *Cryptosporidium parvum* transcriptome during in vitro development. *PloS ONE*, **7**, e31715.

Ming, Z., Y. Wang, A. Y. Gong, X. T. Zhang, M. Li, T. Chen, N. W. Mathy, J. Strauss-Soukup & X. M. Chen, 2018. Attenuation of intestinal epithelial cell migration during *Cryptosporidium parvum* infection involves parasite Cdg7_FLc_1030 RNA-mediated induction and Release of Dickkopf-1. *The Journal of Infectious Diseases*, **218**, 1336–1347.

Muñoz-Caro, T., M. Lendner, A. Daugschies, C. Hermosilla & A. Taubert, 2015. NADPH oxidase, MPO, NE, ERK1/2, p38 MAPK, and Ca2+ influx are essential for *Cryptosporidium parvum*-induced NET formation. *Developmental & Comparative Immunology*, **52**, 245–254.

O'Connor, R. M., P. B. Burns, T. Ha-Ngoc, K. Scarpato, W. Khan, G. Kang & H. Ward, 2009. Polymorphic mucin antigens CpMuc4 and CpMuc5 are integral to

Cryptosporidium parvum infection *in vitro*. *Eukaryotic Cell*, **8**, 461–469.

Pawlowic, M.C., M. Somepalli, A. Sateriale, G.T. Herbert, A. R. Gibson, G. D. Cuny, L. Hedstrome & B. Striepen, 2019. Genetic ablation of purine salvage in *Cryptosporidium parvum* reveals nucleotide uptake from the host cell. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 21160–21165.

Singh, P., B. R. Mirdha, A. Srinivasan, L. A. Rukmangadachar, S. Singh, P. Sharma & K. Luthra, 2015. Identification of invasion proteins of *Cryptosporidium parvum*. *World Journal of Microbiology and Biotechnology*, **31**, 1923–1934.

Wang,Y., Y. Shen, H. Liu, J. Yin, X-T. Zhang, A-Y Gong, X. Chen, S. Chen, N. W. Mathy, J. Cao & X-M. Chen, 2019. Induction of inflammatory responses in splenocytes by exosomes released from intestinal epithelial cells following *Cryptosporidium parvum* infection. *Infection and Immunity*, **87**, e00705–e00718.

Yasur-Landau, D., M. Zilberberg, M. P. Markovich, A. Behar, L. Fleiderovitz & M. L. Mazuz, 2021. *Cryptosporidium parvum* subtypes from diarrheic dairy calves in Israel. *Veterinary Parasitology: Regional Studies and Reports*, **25**, 100608.

Zaalouk, T. K., G. A. Abo Sheishaa & I. R. Shalash, 2020. Downregulation of toll-like receptor 4 and acute phase cytokines mediated dexamethasone immunosuppressant mechanism during *Cryptosporidium-parvum* infection. *Journal of the Egyptian Society of Parasitology*, **50**, 197–202.

Paper received 12.09.2022; accepted for publication 16.11.2022

Correspondence:

Shapoor Reza Shojaei
Department of Parasitology,
Karaj Branch,
Islamic Azad University,
Karaj, Iran
e-mail: sshojaei@kiau.ac.ir